

Title (en)
NOVEL GENE TARGETING METHOD

Title (de)
NEUARTIGES GEN-TARGETING-VERFAHREN

Title (fr)
NOUVEAU PROCÉDÉ DE CIBLAGE DE GÈNE

Publication
EP 3290518 A4 20181205 (EN)

Application
EP 16785983 A 20160429

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Abstract (en)
[origin: EP3290518A1] Provided are a novel two-step gene targeting method and a nucleotide construct for gene targeting. The method can improve the gene targeting efficiency and accurately identify a target gene knock-out mutant. The method of the present invention comprises: firstly, efficiently replacing a target gene in a genome with a targeting box by homologous recombination, the targeting box consisting of a target gene activity variant, a marker gene and site-specific recombination sites; and secondly, resecting the targeting box by recombinase, leaving a site-specific recombination site on the target gene to generate a target gene knock-out mutant, and removing a recombinase expression vector from the knock-out mutant by using a counter selection marker in the recombinase expression vector.

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Citation (search report)
• [XY] HEANEY JASON D. ET AL.: "Tissue-specific expression of a BAC transgene targeted to the Hprt locus in mouse embryonic stem cells", GENOMICS, vol. 83, no. 6, 2004, pages 1072 - 1082, XP029302978, ISSN: 0888-7543, DOI: 10.1016/J.YGENO.2003.12.015
• [XY] HERNANDEZ DIANA ET AL.: "Deletion of genes from the mouse genome using Cre/loxP technology", METHODS IN MOLECULAR BIOLOGY (CLIFTON, N.J.) 2006, vol. 320, 2006, pages 307 - 319, XP009508620, ISSN: 1064-3745
• [Y] JOHANSSON B. AND HAHN-HÄGERDAL B.: "Overproduction of pentose phosphate pathway enzymes using a new CRE-loxP expression vector for repeated genomic integration in Saccharomyces cerevisiae", YEAST, JOHN WILEY & SONS LTD, GB, vol. 19, no. 3, 1 February 2002 (2002-02-01), pages 225 - 231, XP002399562, ISSN: 0749-503X, DOI: 10.1002/YEA.833
• [A] STEPHAN J. ET AL.: "Consecutive gene deletions in Mycobacterium smegmatis using the yeast FLP recombinase", GENE, ELSEVIER, AMSTERDAM, NL, vol. 343, no. 1, 8 December 2004 (2004-12-08), pages 181 - 190, XP004653653, ISSN: 0378-1119, DOI: 10.1016/J.GENE.2004.08.028
• See references of WO 2016173555A1

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